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Regulation of Nitrogen Uptake and Assimilation: Effects of Nitrogen Source, Root-Zone pH, and Aerial CO₂ Concentration on Growth and Productivity of Soybeans

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PROJECT SUMMARY

The present knowledge of whole-plant regulation of nitrogen absorption and utilization from NO_3^- , NH_4^+ or mixed NO_3^- and NH_4^+ nitrogen sources during vegetative and reproductive growth is inadequate to formulate decisions on the most effective nitrogen supply for efficiency of plant productivity. Root-zone and aerial environments influence growth and yield responses of plants grown with these nitrogen sources through their interactive effects on physiological processes. Planning for efficient utilization of nitrogen supply to support yield of a controlled-environment crop production system for CELSS requires a better understanding of the relationship between nitrogen source and the physiological processes which regulate vegetative and reproductive plant growth.

The overall objective of the proposed research is to characterize regulation of nitrogen uptake and assimilation from NO_3^- and NH_4^+ sources. We will examine the effects of root-zone pH and aerial CO_2 concentration on uptake and utilization of the two nitrogen forms during vegetative and reproductive growth. These two environmental factors are of particular interest due to their differential effects on NO_3^- and NH_4^+ uptake rates and subsequent assimilation into reduced organic nitrogen compounds, and the interdependence of these processes on photosynthetic rate and photosynthate partitioning.

Results of research completed during the past year and the research direction for the upcoming year are summarized in the following pages.

SUMMARY OF RESEARCH DURING CURRENT YEAR OF FUNDING

1. JUSTIFICATION AND OBJECTIVES

1.1 Overall objectives.

The research objectives during the current year of funding were (a) to continue experiments on effects of root-zone pH on preferential utilization of NO_3^- versus NH_4^+ nitrogen, (b) to investigate processes at the whole-plant level that regulate nitrogen uptake, (c) to continue investigation of effects of atmospheric CO_2 and NO_3^- supply on growth of soybean, and (d) to examine the role of NO_3^- uptake in enhancement of root respiration.

1.2 Effects of root-zone pH on preferential utilization of nitrate versus ammonium nitrogen.

Utilization of a mixed NO_3^- and NH_4^+ nitrogen source apparently is dependent upon the species involved. Numerous plant species exhibit an enhancement of growth when both nitrogen forms are present in the culture medium (9,18,19,64,82). Yet, the effects of a mixed nitrogen source on growth and rate of uptake of nitrogen depend on the ratio of the two ions present and on the total nitrogen concentration (26, 58). Although numerous studies have been reported on this subject, the specific effects of mixed nitrogen source on absorption and utilization of individual nitrogen forms remain vague. In some cases, antagonism, or direct inhibition of absorption of NO_3^- by NH_4^+ (16,17,44,45,58), or inhibition of NH_4^+ uptake by NO_3^- (82) has been reported. It is likely that these results are dependent upon the precision of pH control of the culture medium (83). In

other cases, there is no direct effect of NH_4^+ on NO_3^- uptake, yet NO_3^- assimilation is reduced (41,64,65). The physiological reasons for the wide range of responses are still unclear, though numerous hypotheses have been suggested (26).

We have been investigating the pattern of nitrogen uptake and assimilation in soybeans grown with mixed NO_3^- and NH_4^+ nitrogen in solution culture. Specifically, we are studying whether one form of nitrogen is preferentially utilized to supply nitrogen during vegetative growth by simultaneously monitoring uptake of both nitrogen species from solution using ion chromatography. In addition, we have been examining the influence of solution pH on the preferential use of these two nitrogen forms. We hypothesize that the accelerated uptake of one form of nitrogen at low or high pH will compensate for the nitrogen deprivation which occurs as a result of decreased rate of uptake of the other nitrogen source at the corresponding solution pH.

1.3 Whole-plant regulation of nitrogen uptake.

In studies with nonnodulated soybean plants growing in flowing solution culture, the net rate of NO_3^- uptake during vegetative growth cycles between maxima and minima with a periodicity of oscillation of 3 to 5 d (70,74). We have proposed that this fluctuation in net rate of NO_3^- uptake occurs in response to changes in demand for carbon and nitrogen in the shoot and the availability of carbohydrate for translocation from shoot to roots associated with periodic emergence and expansion of new leaves. This conclusion is based on observations

that NO_3^- uptake and transport are dependent on respiratory energy (53), soluble carbohydrate status of roots generally is low (55,57) so that continued absorption of NO_3^- depends on continued supply of carbohydrate from the shoot for respiratory energy (31), and translocation of carbohydrate from shoot to roots is responsive to concentration of carbohydrate in the shoot pool and to size and metabolic activity of root sinks (81). As nitrogen is absorbed by roots and translocated to the shoot, stimulation of shoot growth and initiation and early expansion of new leaves (71,72) presumably reduces the availability of carbohydrate for translocation to roots as metabolic demand of the shoot is increased more rapidly than photosynthetic capacity. The reduced flow of carbohydrate to roots results in a decrease in NO_3^- uptake. The subsequent reduction in nitrogen availability to the shoot decreases initiation and expansion of new leaf tissue before reducing photosynthetic activity of the shoot (71,72); thus, availability of carbohydrate for transport to roots, and consequently to support NO_3^- uptake, increases. From these observations, net rates of NO_3^- uptake should fluctuate with a periodicity similar to the interval between emergence of new leaves, which at about 3 to 4 d for soybean (68,72,74) corresponds to the observed periodicity in cycles of NO_3^- uptake (70,74), and with the minima of net uptake coordinated with time of leaf emergence and early expansion.

When acidity of the root-zone is controlled at pH 6.0 in solution culture, many plants are equally effective in utilization of NH_4^+ or

NO_3^- as a nitrogen source with no differences in nitrogen accumulation or growth (73). This indicates that, while fluxes of NO_3^- and NH_4^+ into roots are facilitated by separate, inducible transporters (22,29,30) and while NH_4^+ is assimilated almost exclusively in roots (20,40) but NO_3^- is assimilated in both roots and leaves or stored in vacuoles (4,24,59), regulation of NH_4^+ uptake at the whole-plant level may involve the same mechanism as regulation of NO_3^- uptake. If so, net uptake rate of NH_4^+ would demonstrate oscillations with a periodicity coordinated with the interval of leaf emergence. The objective of this experiment was to determine whether such cyclic variations occur in uptake rates of NH_4^+ under environmental conditions that do not otherwise impede NH_4^+ uptake.

1.4 Effects of atmospheric carbon dioxide and nitrate supply on growth of soybean.

Elevated concentrations of atmospheric CO_2 generally enhance growth of crop plants (1,2), with the magnitude of the response dependent upon other environmental factors (1,34). Numerous investigations have shown that the increase in dry matter for plants grown with elevated atmospheric CO_2 is accompanied by lower concentrations of mineral nutrients in general, and nitrogen in particular (5,10,21,51,52,66,67,84,85). While increased levels of nitrogen fertilization can enhance growth under elevated CO_2 (5,10,52,84), the question is raised as to whether the capacity of plants to take up and assimilate nitrogen limits the ability to respond to elevated CO_2 .

In assessing the effects of atmospheric CO_2 and nitrogen on plant

growth, one must consider whether uptake and utilization of nitrogen is limited by physiological characteristics of the plant, or by the availability of nitrogen for uptake at the root surface. Most studies on the response to elevated CO_2 have been conducted on plants grown in solid media such as soil material or peatmoss-vermiculite mixtures. In solid media, NO_3^- moves through the unstirred soil solution by diffusion or mass-flow. Although the gradient for movement of NO_3^- through soil solution to the root surface is more favorable as NO_3^- in the rhizosphere is depleted by uptake, the diffusion process is slow relative to the movement via mass-flow when transpiration is rapid (6). Stomatal resistance of plants generally is increased at elevated CO_2 , which results in decreased transpiration (1,32,52). The decreased transpiration, by effectively decreasing movement of solution, could reduce mass-flow of NO_3^- into the rhizosphere in solid medium (56). As a result, available nitrogen at the root surface may become limiting under elevated CO_2 at a concentration in the bulk solution that was adequate at lower CO_2 levels. For plants grown with a solid substrate, it is unclear if the greater requirement for nitrogen fertilization is due to inherent effects of elevated CO_2 on nitrogen uptake and utilization (52), or to changes in nitrogen availability to roots related to the movement of NO_3^- through the culture medium to the rhizosphere.

In flowing-solution culture the availability of nitrogen at the root surface and nitrogen uptake by the root are not dependent on the

mass-flow of solution to the root. If movement of NO_3^- by mass-flow is the limiting factor in nitrogen uptake for plants growing at elevated concentrations of CO_2 in solid substrate, plants under elevated CO_2 in hydroponic culture should not be limited by NO_3^- uptake. In fact, Kencht and O'Leary (33) found no change in nutritional requirements of lettuce grown in hydroponic culture despite the increase in plant growth with elevated atmospheric CO_2 . The objective of this study was to test the hypothesis that in flowing hydroponic culture soybean plants grown at elevated levels of atmospheric CO_2 do not respond to external NO_3^- at a concentration greater than the concentration that supports maximal growth at lower levels of CO_2 . The hypothesis was tested by growing soybean plants at 400, 650, or 900 $\mu\text{L L}^{-1}$ CO_2 in hydroponic solutions containing 0.5, 1.0, 5.0, or 10.0 mM NO_3^- . Previous results (74) have shown that maximum growth rate for nonnodulated soybean plants during vegetative growth under 400 $\mu\text{L L}^{-1}$ CO_2 is supported by 0.5 mM NO_3^- in flowing-solution culture and that total nitrogen accumulation in the plants was not affected by increases in solution concentration of NO_3^- to 10.0 mM.

1.5 Enhancement of root respiration during nitrate uptake.

Ion uptake has been proposed as a major sink for respiratory energy in plant roots. The proportion of total root respiration used for ion uptake has been estimated by multiple regression analysis to vary between 20 and 70% for corn (76,77,78) and between 10 and 38% and 10 and 24% for Carex diandra Schrank and Carex acutiformis Ehrh., respectively (75), depending on age and environmental conditions. Recent evidence (23) indicates that not all of the increased

respiration rate for roots actively absorbing NO_3^- is attributable directly to NO_3^- uptake. In two split-root hydroponic systems using maize plants with two primary nodal roots, the rate of NO_3^- uptake per unit root weight was 1.6 times greater for an axis exposed to exogenous NO_3^- when the other axis was not exposed to NO_3^- than to either axis when both were exposed to NO_3^- . The specific rates of respiration (respiration rate per unit root dry weight) for the +N axes in these two split-root systems were relatively unaffected by their rates of NO_3^- uptake. Thus, although part of the increased respiration for roots absorbing NO_3^- was related to the energy requirement for uptake, in situ reduction, and translocation of NO_3^- , other components of respiration also were affected.

An alternative supposition for the enhancement in specific rate of respiration for roots exposed to exogenous NO_3^- (23,37) is increased metabolic activity that would be associated with the proliferation of lateral branches (13,14,15,23). Proliferation of lateral branches is positively associated with the concentration of reduced nitrogen in the axis (23). Although cycling of nitrogen between shoot and roots supplies reduced nitrogen to all portions of the root system (8,23,79,86), in situ reduction and utilization of NO_3^- contribute to a greater concentration of reduced nitrogen in axes actively absorbing NO_3^- than in axes growing in the absence of NO_3^- (23). Roots entirely dependent upon recycling from the shoot for reduced nitrogen continue to elongate, but initiation of lateral branches is restricted (15,23, 25). Since specific rate of respiration for excised root apices is greater than for more basal regions (60), the increased production of

apices stimulated by absorption and in situ utilization of NO_3^- (23) should contribute to total respiration of roots disproportionately to the uptake of NO_3^- .

These observations suggest a positive relationship between concentration of reduced nitrogen in roots and the extent of proliferative, versus elongative, growth and hence between concentration of reduced nitrogen and specific rate of respiration of roots. Both of these factors would be enhanced for portions of a root system actively absorbing NO_3^- . To evaluate the validity of these relationships, three conditions for supply of reduced nitrogen to roots can be established. At one extreme, if exogenous NO_3^- is withheld from both axes of a split-root system, the supply of reduced nitrogen to roots will be dependent entirely upon recycling from a nonreplenished pool in the shoot. A second condition for supply of reduced nitrogen can be established by supplying exogenous NO_3^- to only one of the two axes in the split-root system. Recycling from the shoot still would be the only source of reduced nitrogen for the -N axis of this pair, but the shoot pool would be replenished continuously with nitrogen absorbed and translocated from the +N axis. Axes that are directly exposed to exogenous NO_3^- during treatment provide a third condition for supply of reduced nitrogen in which the source of reduced nitrogen would include in situ reduction of absorbed NO_3^- in addition to recycling from a continually replenished shoot pool. Since estimates of the proportion of root respiration utilized for ion uptake reportedly (76,77) are influenced by light-limited availability of photosynthates

for translocation from shoot to roots, variation in irradiance potentially would modify the responses between concentration of reduced nitrogen and specific rate of respiration within these conditions for supply of reduced nitrogen for roots.

2. PROCEDURES AND RESULTS

2.1 General procedures.

Experiments were conducted in the controlled-environment growth facilities of the phytotron at North Carolina State University. Soybean, one of the potential species proposed for a crop production system of CELSS, was the species used in all experiments except those to examine the role of NO_3^- uptake in enhancement of root respiration. Maize was utilized in these latter experiments. Plants were grown hydroponically in temperature-controlled, continuous-flow culture systems equipped with automated pH control (80). Unless otherwise stated, plants were grown at aerial day/night temperatures of 26/22°C, root-zone temperature of 24°C, PPFD (photosynthetic photon flux density) of $700 \mu\text{mol m}^{-2}\text{s}^{-1}$ from a combination of fluorescent and incandescent lamps during a 9-h light period, an aerial CO_2 concentration of $400 \mu\text{mol L}^{-1}$, and root-zone pH of 6.0 (80). Acidity of complete nutrient solutions was controlled by automatic additions of 0.01 N H_2SO_4 and $\text{Ca}(\text{OH})_2$. Floral development of soybean was repressed by a 3-h interruption during the middle of the 15-h dark period with incandescent lamps (68). All experiments included a pretreatment period of 14 to 16 d before initiation of treatments. Nitrogen concentrations in nutrient solutions were monitored daily by ion

chromatography and NO_3^- or NH_4^+ was resupplied to return solutions to their initial concentrations. Net uptake of NO_3^- or NH_4^+ was determined as daily depletion from the replenished solutions. To avoid depletion of other nutrients, half of the nutrient solution in the hydroponic systems was replaced every 3 or 4 d.

Plants were sampled at 2- to 4-d intervals for destructive measurements of dry weight, leaf area, and nitrogen content. Total nitrogen in tissues was determined by a micro-Kjeldahl procedure which included a predigestion in salicylic acid to aid in the recovery of NO_3^- -N. Concentrations of NO_3^- -N in tissues were determined chromatographically in hot water extracts of freeze-dried tissue. Reduced nitrogen in tissues was determined as the difference between total nitrogen and NO_3^- -N.

For measurement of root respiration, maize plants with two primary nodal root axes were grown in a split-root hydroponic system (23) in which NO_3^- was supplied to both axes at 1.0 mM, to one axis at 1.0 mM and the other axis at 0.0 mM, or to both axes at 0.0 mM. Respiration of each axis was measured at 2-d intervals as the rate of CO_2 evolution from attached roots in a Plexiglas respirometer containing the same nutrient solution in which the roots had been growing (23).

2.2 Effects of root-zone pH on preferential utilization of nitrate versus ammonium nitrogen.

A series of experiments have been completed in which soybean

plants were cultured with nitrogen sources of NO_3^- , NH_4^+ , or mixed NO_3^- and NH_4^+ and at a range of pH between 6.0 and 4.5. During a 16-d pretreatment period, soybean seedlings were cultured on complete nutrient solutions containing 1.0 mM NO_3^- and a controlled pH level of 6.0. During a 21-d treatment period, plants were cultured on complete nutrient solutions which contained a total nitrogen concentration of 1.0 mM and which had the following $\text{NO}_3^-/\text{NH}_4^+$ ratios: 1.0/0.0, 0.67/0.33, 0.33/0.67, and 0.0/1.0. These treatments were repeated in experiments carried out with pH of the culture solutions controlled at 6.0, 5.5, 5.0, and 4.5 during the treatment periods.

There were no differences in accumulation of dry matter by plants at pH levels of 6.0 (Fig. 2.21), 5.5, and 5.0 (data not shown). However, dry matter accumulation by plants cultured on 1.0 mM NH_4^+ was less than for the other nitrogen source treatments at pH 4.5 (Fig. 2.21). Total nitrogen accumulation by plants cultured on 1 mM NH_4^+ also was significantly lower than plants cultured on the other nitrogen source treatments at pH 4.5 (Fig. 2.22). These data support our hypothesis that plants grown at low pH with NH_4^+ as the sole source of nitrogen undergo nitrogen deficiency due to the unfavorable proton gradient between root tissue and external solution for the absorption of NH_4^+ (73).

Plants which were exposed to mixed NO_3^- and NH_4^+ sources at pH 6.0 showed a preference for NH_4^+ as a nitrogen source by a ratio of 2:1 over NO_3^- , regardless of which nitrogen ion was in greater abundance

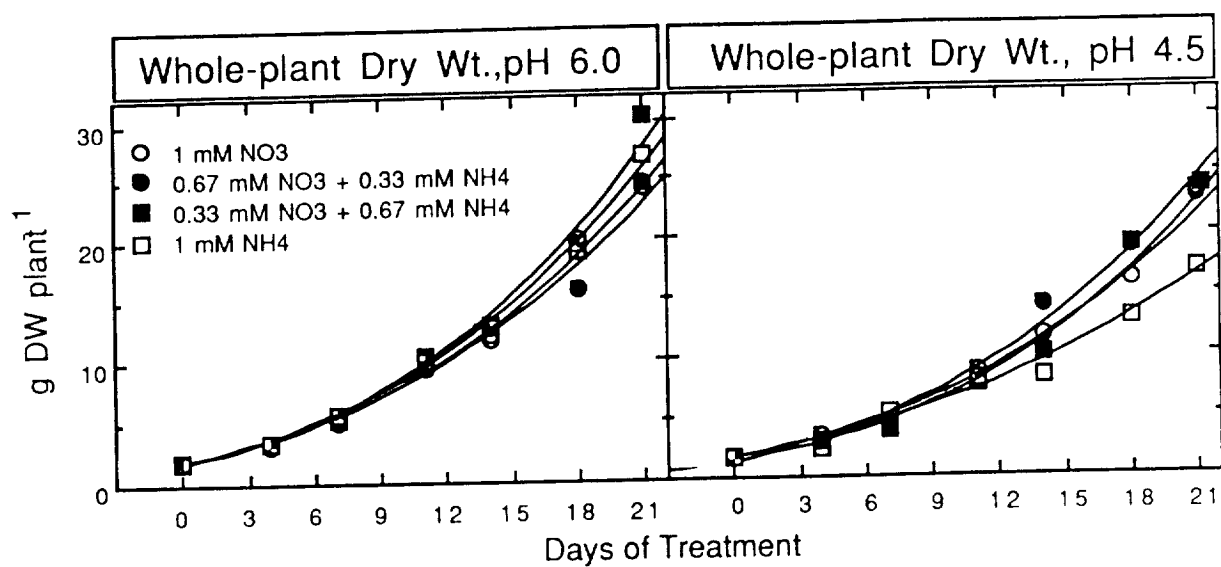


FIGURE 2.21 Dry matter accumulation by plants cultured on nitrate, ammonium, or mixed nitrate and ammonium sources at pH 6.0 or 4.5.

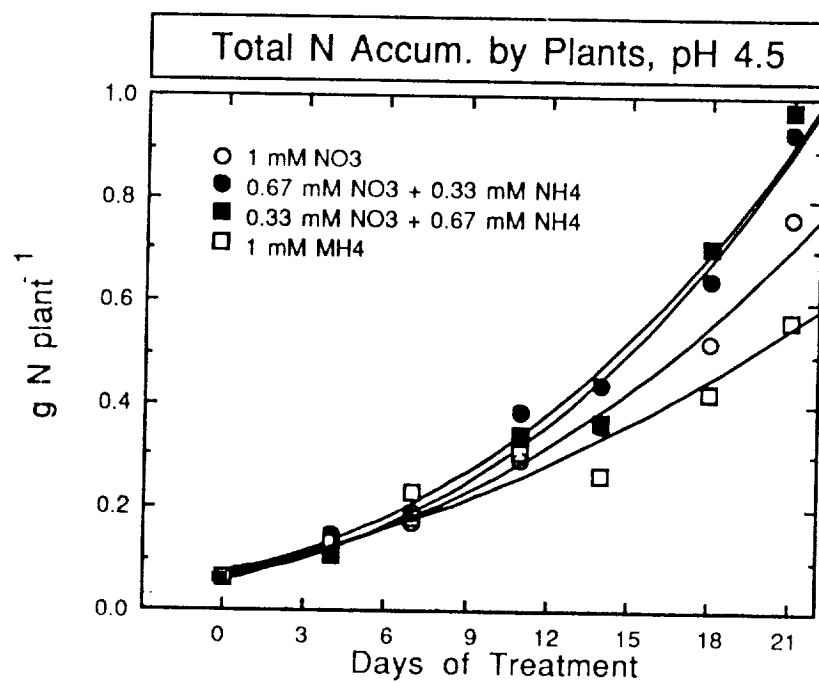


FIGURE 2.22. Total nitrogen accumulated by plants cultured on nitrate, ammonium, or mixed nitrate and ammonium sources at pH 4.5.

(Table 2.21). However, the relative uptake of $\text{NH}_4^+:\text{NO}_3^-$ by the plants declined as pH of the nutrient solutions decreased until, by pH 4.5, slightly more NO_3^- than NH_4^+ was absorbed by plants in the 0.67 mM NO_3^- plus 0.33 mM NH_4^+ treatment (Table 2.21). These observations also support our hypothesis that conditions for NH_4^+ absorption become less favorable as pH declines. Also note that below pH 6.0 there is an apparent effect of the relative abundance of NO_3^- and NH_4^+ on the ratio of their absorption from the mixed nitrogen sources (Table 2.21).

Plants which were supplied with mixed NO_3^- and NH_4^+ sources generally accumulated more total nitrogen in their tissues at each pH treatment than plants which were exposed solely to NO_3^- or NH_4^+ sources (Table 2.22, Fig. 2.22). Analysis of tissue NO_3^- concentrations (data not shown) suggests that the increased concentration of total nitrogen in these tissues may be a result of vacuolar storage of inorganic NO_3^- (24).

2.3 Whole-plant regulation of nitrogen uptake.

Based on previous investigation (70,74), we have proposed that net uptake of nitrogen from flowing solution culture fluctuates during vegetative growth in response to changes in demand for carbon and nitrogen in the shoot and availability of carbohydrate for translocation from shoot to roots associated with periodic emergence and expansion of new leaves. The sole nitrogen source for soybean plants in these prior experiments was NO_3^- . Since soybean plants can utilize NH_4^+ equally as effectively as NO_3^- when acidity of the solution is maintained near pH 6.0 (73), we further have proposed that, although

TABLE 2.21 Ratio of ammonium to nitrate uptake by soybean plants from complete nutrient solutions containing nitrate and ammonium and controlled at pH 6.0, 5.5, 5.0, or 4.5 over a 21-d period.

pH	Treatments	
	0.67 mM NO_3^- + 0.33 mM NH_4^+	0.33 mM NO_3^- + 0.67 mM NH_4^+
	$(\text{NH}_4^+:\text{NO}_3^-)$	
6.0	1.9	2.1
5.5	1.2	1.9
5.0	1.2	1.9
4.5	0.9	1.5

TABLE 2.22 Total nitrogen content of soybean plants cultured on complete nutrient solutions containing nitrate, ammonium, or mixed nitrate and ammonium sources of nitrogen and maintained at pH 6.0, 5.5, 5.0, or 4.5 for 21 d.

pH	Treatments			
	1.0 mM NO_3^-	0.67 mM NO_3^- + 0.33 mM NH_4^+	0.33 mM NO_3^- + 0.67 mM NH_4^+	1.0 mM NH_4^+
	(g N plant^{-1})			
6.0	0.879	1.101	1.298	1.003
5.5	0.986	1.057	1.230	1.050
5.0	0.892	0.955	1.118	1.008
4.5	0.766	0.932	0.974	0.565

fluxes of NH_4^+ and NO_3^- into roots are facilitated by separate transporters (22,29,30) and their patterns of transport and assimilation within the plant are distinct (4,20,24,40,59), regulation of NH_4^+ and NO_3^- uptake at the whole-plant level should behave similarly in response to shifting demands for carbon and nitrogen during leaf emergence and expansion.

In the present experiment with nonnodulated soybean, root temperature was maintained at 24°C and aerial temperature was changed from 26/22°C during pretreatment to 18/14°C during the treatment period. Dry matter accumulation in shoot and root tissues (Fig. 2.31A) and nitrogen accumulation by the whole plant (Fig. 2.31B) during a 21-d period of vegetative growth in which NH_4^+ was the sole nitrogen source were quantitatively similar to that observed previously (70,71,74) when NO_3^- was the sole nitrogen source. Cumulative depletion of NH_4^+ -N from the replenished solutions over the treatment period was in close agreement with total nitrogen accumulation in plants as determined by tissue analysis (Fig. 2.31B). Net rate of NH_4^+ uptake per g root dry weight (Fig. 2.32B), as calculated from daily depletion from solution per plant (Fig. 2.32A) and predicted root dry weights (Fig. 2.31A), cycled between maxima and minima during the treatment period. Thus, the oscillations in net rate of uptake that have been observed for NO_3^- (7,74) also occur for NH_4^+ .

If, as proposed (70,74), the fluxuations in uptake rate of nitrogen are a response to changes in flux of carbohydrate from shoot to root, and the availability of carbohydrate for transport to roots

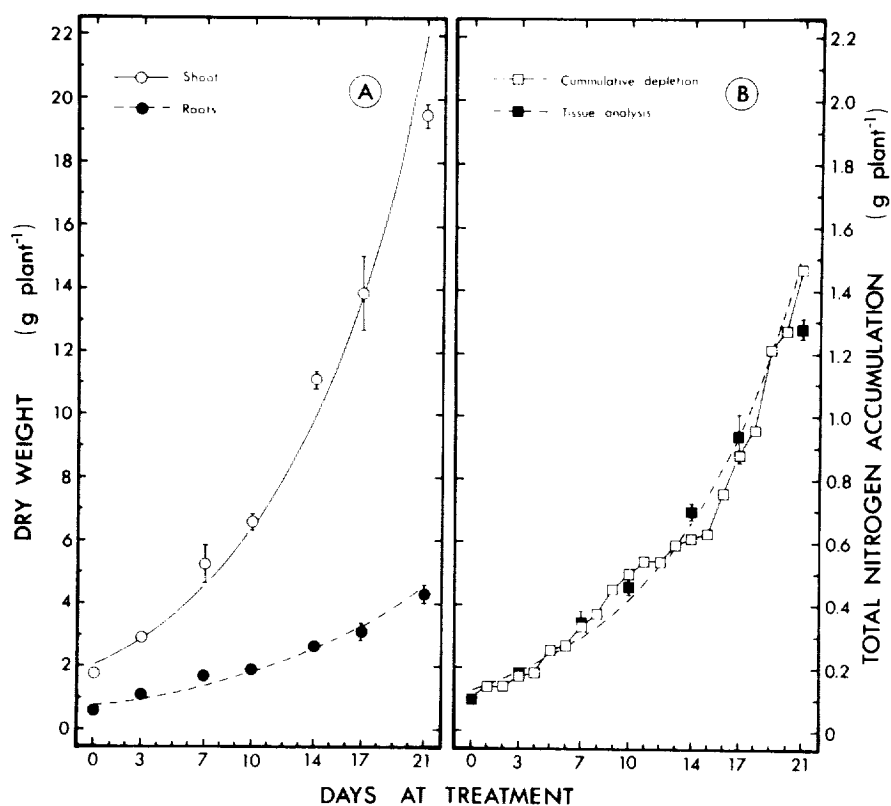


FIGURE 2.31 Dry matter accumulation in shoot and roots (A) and total nitrogen accumulation as determined by cumulative depletion of NH_4^+ from replenished solution and by analysis of tissues (B). Each value is the mean of three plants with vertical bars representing ± 1 s.d. of the mean when greater than the size of the symbol. Regression equations relating grams of dry weight for shoot (DW_S) and roots (DW_R) and total nitrogen in plants (N) to days (d) treatment are: $\ln \text{DW}_S = 0.114(d) + 0.714$, $r = 0.99$; $\ln \text{DW}_R = 0.087(d) - 0.280$, $r = 0.98$; $\ln N = 0.118(d) - 2.065$, $r = 0.99$.

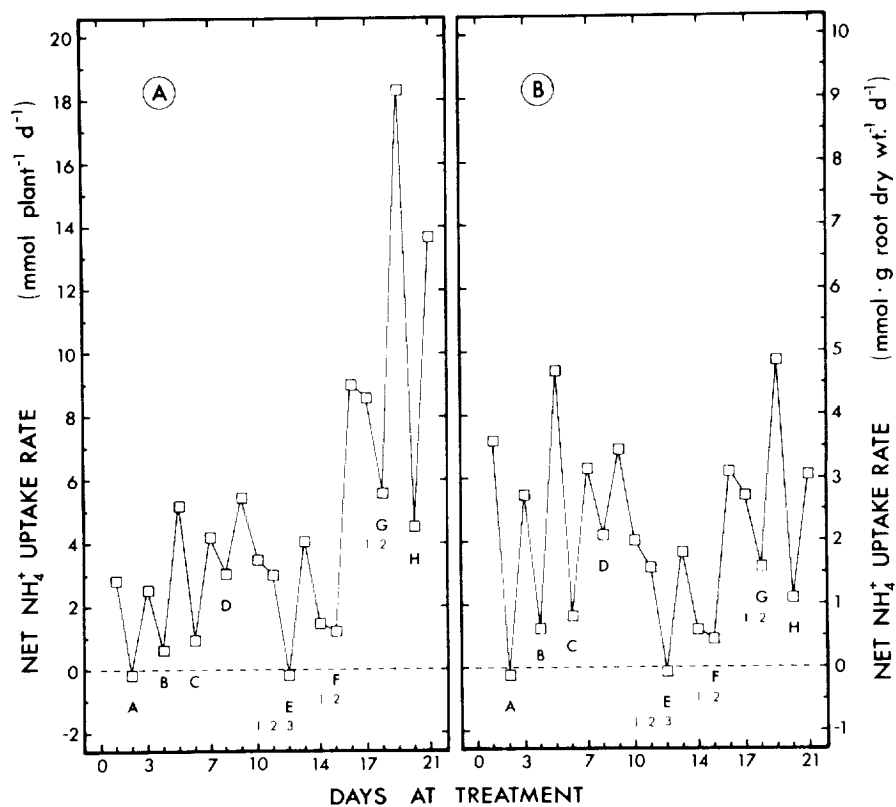


FIGURE 2.32 Net rate of NH_4^+ uptake per plant calculated from daily depletion of NH_4^+ from replenished solution (A) and net rate of NO_4^+ uptake per gram root dry weight (B) calculated from daily depletion per plant and root dry weights estimated from regression equation in Fig. 2.31A. Minima in net uptake rate are labeled by upper case letters, and shoulders on the down slope of minima E, F, and G are labeled in numerical order for identification in Fig. 2.34.

declines during initiation and early expansion of new leaves (55,57,81), the intervals between occurrence of uptake minima and emergence and early expansion of new leaves should be coincident. Since leaves are initiated on both the mainstem and axillary branches of soybean plants, synchronous emergence of groups of leaves among the multiple stems must occur for the proposed relationship to be tenable. In the present experiment, trifoliolate leaves on the mainstem and on individual axillary branches were counted at each harvest (Fig. 2.33). Since plants were harvested at 3- to 4-d intervals, the most recently emerged leaves were at variable stages of development and ranged from 2 to 49 cm² in area when first counted. The rates of emergence of leaves on the main stem (Fig. 2.33A) and on each branch (Fig. 2.33B) thus were estimated by linear regression ($r = 0.96$ or greater), and the intervals of emergence for leaves on each stem were calculated as the reciprocals of these rates. The calculated interval of leaf emergence on the main stem was 2.2 d leaf⁻¹ during the initial 5.6 d of treatment and 3.7 d leaf⁻¹ during the remainder of the study. The change in rate of emergence was accepted as a consequence (68) of the decrease in aerial temperature at the initiation of the treatment period. For branch stems, the interval of leaf emergence ranged from 3.8 to 7.2 d leaf⁻¹. The calculated values were used to reconstruct the time of emergence of each leaf on the plant (Fig. 2.34) to determine if the order of appearance of leaves on the various stems could be indentified into eight groups with mean days of emergence corresponding to the occurrence of the eight minima (A-H,

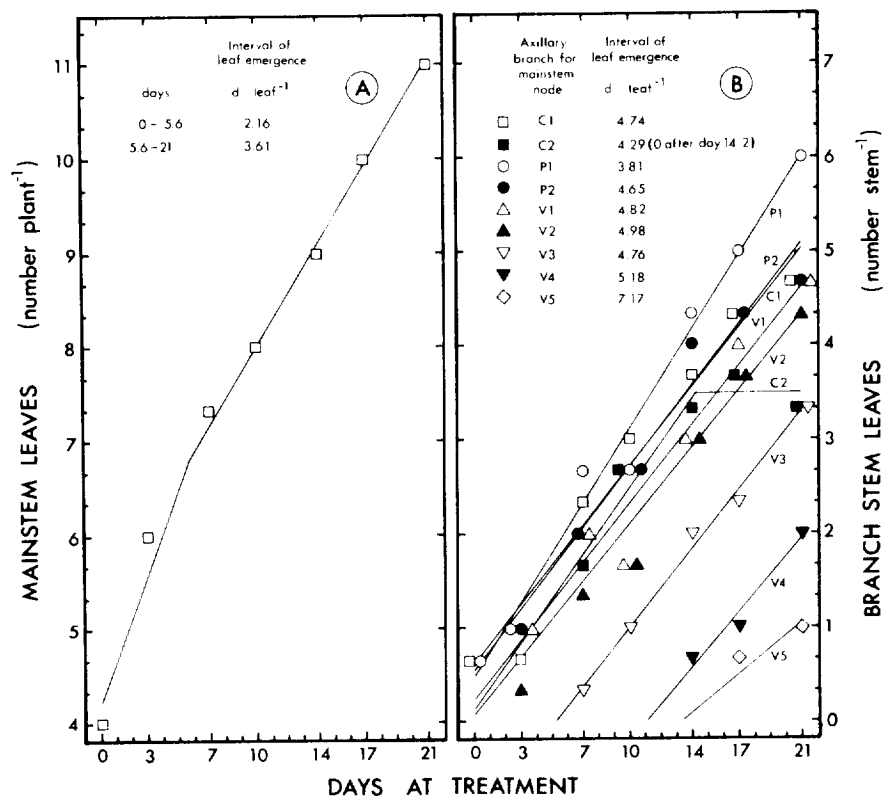


FIGURE 2.33 Number of trifoliolate leaves on the main stem (A) and on each axillary branch (B) of plants at each harvest. Axillary branches are designated as C1 and C2 at the cotyledonary node, P1 and P2 at the primary node, and V1 to V5 at successive acropetally numbered trifoliolate nodes on the main stem. For clarity, overlapping values for sample day are depicted side-by-side. Each value is the mean of three plants. Intervals of leaf emergence for each stem are calculated as reciprocals of the rates of leaf appearance derived as the slope of linear regression equations relating number of leaves to days of treatment.

Fig. 2.32) in net rate of NH_4^+ uptake.

The mean days of emergence for the eight groups identified (A'-H', Fig. 2.34) were 1.9 (A'), 4.0 (B'), 6.4 (C'), 8.2 (D'), 10.6 (E'), 13.9 (F'), 16.8 (G'), and 20.4 (H') d. The mean days of emergence for these eight groups were highly correlated ($r=0.99$) with the occurrence of net uptake minima at 2, 4, 6, 8, 12, 15, 18, and 20 d (inset, Fig. 2.34). The largest standard deviations of the mean day of emergence, as well as the greatest differentials between mean day of emergence and time of observed minima net rate of NH_4^+ uptake, occurred for leaf groups E', F', and G'. The contours of the slopes in uptake rate between the minima for these leaf groups and the preceeding maxima had one or two shoulders (Fig. 2.32). If the leaves within these groups are divided into subgroups e1, e2, and e3 for group E', f1 and f2 for group F', and g1 and g2 for group G' (Fig 2.34), the mean days of emergence for the subgroups closely correspond (inset, Fig. 2.34) with the occurrence of each shoulder and the nadir of the declines in uptake rate. These results are consistent with the hypothesis that the periodicity of oscillations in net uptake rate of NH_4^+ , as apparently also was the case for oscillations in net uptake rate of NO_3^- , is coincident with times of emergence of discrete groups of leaves, and thus is coordinated with flux of carbohydrate from shoot to root.

While the periodicity in net uptake rate of nitrogen may reflect changes in translocation rates of carbohydrates from shoot to roots, shifts in the influx and/or efflux components of NO_3^- uptake (11,38,39,42) have been implicated (74) in determining the amplitudes

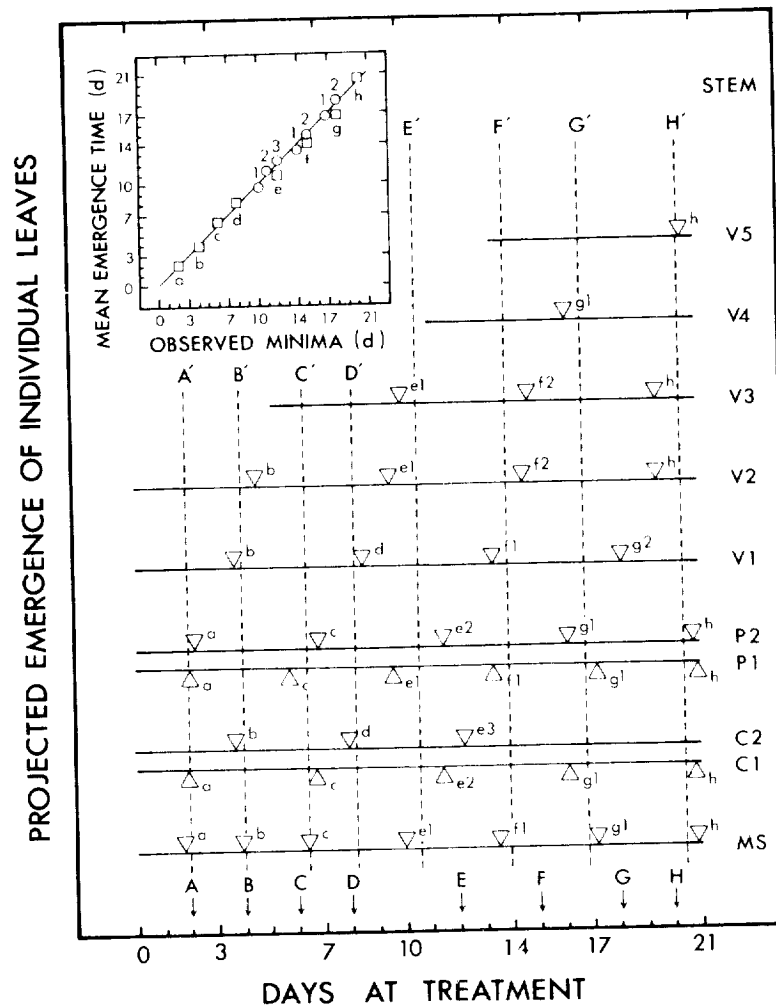


FIGURE 2.34 Estimated time of emergence of leaves on the main stem and axillary stems for cotyledonary (C1 and C2), primary (P1 and P2), and main stem trifoliolate (V1 - V5) nodes based on calculated intervals of leaf emergence from Fig. 2.33. Stems are represented as solid horizontal lines and time of appearance of a leaf as a triangle. Dashed vertical lines are the mean day of emergence for leaf groups A' - H', with each of these leaf groups identified with times (A - H) of observed minima in net uptake rate of NH_4^+ from Fig. 2.32. Lower case letters denote individual leaves assigned to each leaf group. Leaves denoted as e1, e2, e3, f1, f2, g1, and g2 represent subgroups within leaf groups E', F', and G' that are associated with the shoulders and nadir of declining uptake rates as denoted in Fig. 2.32. Inset shows the relationship between mean time for emergence of leaves within each group (squares) and subgroup (circles) and time of observed minima and shoulder in uptake rate (see text for further discussion).

of the maxima and minima of oscillations in net rates of NO_3^- uptake. There is evidence of independently regulated influx and efflux components of net NH_4^+ uptake (46,47,48). The influx and efflux components of NH_4^+ uptake were not directly estimated in the present study. The two instances of negative net rates of NH_4^+ uptake at days 2 and 12 (minima A and E, Fig. 2.32), however, are evidence that efflux was occurring as a component in a balance between influx and efflux which determined the amplitude of oscillations in net NH_4^+ uptake. The apparent involvement of influx and efflux components in determining the amplitude of oscillations in net NH_4^+ uptake rates in response to changes in flux of carbohydrate from shoot to roots thus is consistent with the supposition that regulation of NH_4^+ uptake involves mechanisms at the whole-plant level which are similar to those regulating NO_3^- uptake.

2.4 Effects of atmospheric carbon dioxide and nitrogen supply on growth of soybean.

Increasing the concentration of atmospheric CO_2 from 400 to 650 or 900 $\mu\text{L L}^{-1}$ increased dry weight (Table 2.41, Fig. 2.41) of soybean plants during the 18-d N-treatment period, which followed a 16-d pretreatment period during which the plants received 1.0 mM NO_3^- in a complete nutrient solution. The percentage increase in dry matter, however, generally was greatest early in the N-treatment period, and RGR (relative growth rate) during the N-treatment actually declined with increasing CO_2 concentrations. In fact for seedlings transplanted into a 900 $\mu\text{L L}^{-1}$ CO_2 environment, the increase

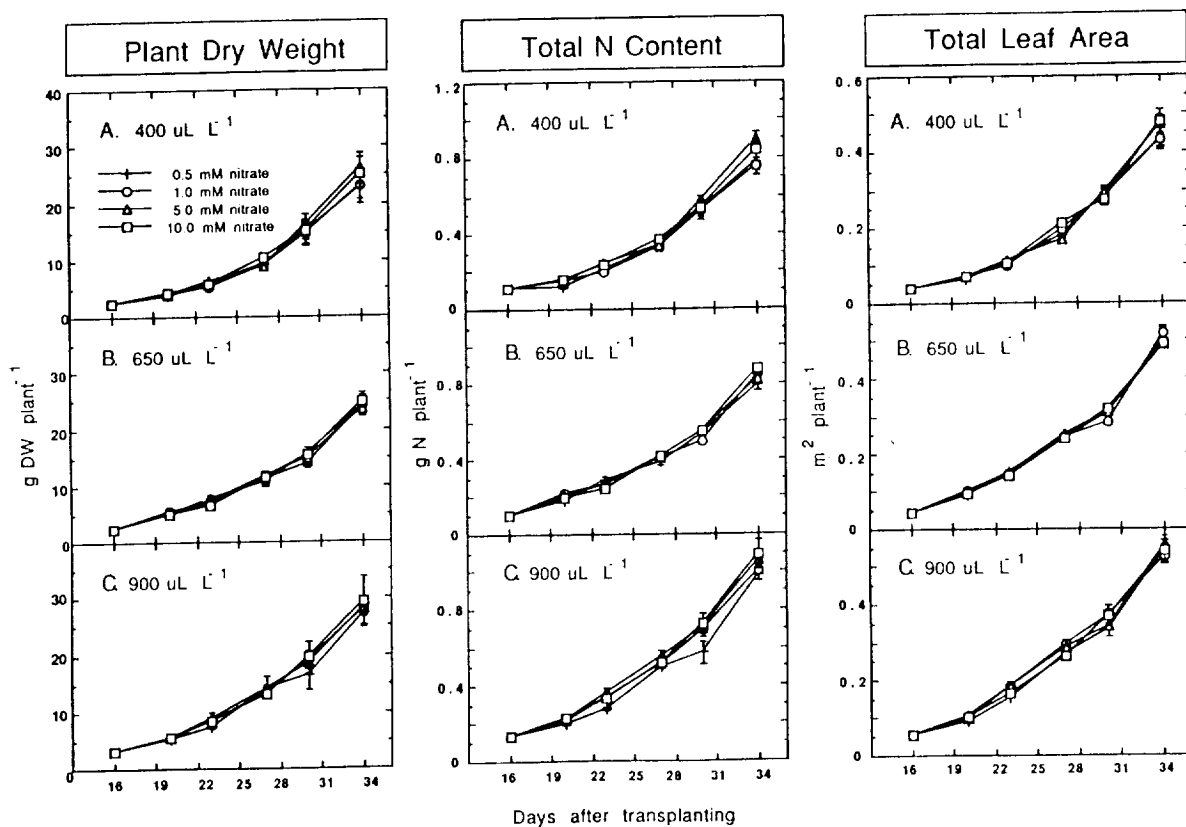


FIGURE 2.41 Plant dry weight, total nitrogen content, and total leaf area of soybean plants grown at CO₂ concentrations of 400, 650, or 900 $\mu\text{L L}^{-1}$ and at nitrate concentrations of the nutrient solutions of 0.5, 1.0, 5.0, or 10.0 mM for an 18-d period. Bars indicate ± 1 s.e.; $n = 3$ for each datum point.

in dry weight was well established within 3 to 7 d during the pre-N-treatment period (Table 2.42). The stimulation in dry matter accumulation took longer for plants exposed to $650 \mu\text{L L}^{-1} \text{CO}_2$, which was not significant until 4 d into the N-treatment period (Table 2.41). Such influences of CO_2 concentration on early growth, followed by a decline in RGR, have been reported previously (28,43,49,69) and identify juvenile growth as a critical period for the influence of CO_2 on increases in dry matter accumulation.

Increasing atmospheric CO_2 level also affected dry matter partitioning and growth within the shoot. Dry matter accumulation (Fig. 2.42C, 2.42E), leaf area (Table 3), and stem length (data not shown) of branch stems of plants grown at elevated CO_2 concentrations were greater early in the N-treatment period, but these differences declined during the period. By day 14, there were no significant differences in these branch stem parameters among CO_2 treatments. Treatment effects of CO_2 concentration were apparent on dry matter accumulation (Fig. 2.42B, 2.42D), leaf area (Table 3), and stem length (data not shown) of the main stem early in the N-treatment period, but not to the same degree observed in the branch stem parameters. However, the increases in these parameters of the main stem were sustained throughout the N-treatment period. These data indicate that meristems of branches responded quicker than the meristem of the main stem, suggesting a greater sensitivity of meristems of branch stems to CO_2 . The similarity of growth parameters of the branch stems by the end of the experimental period (Fig. 2.42C, 2.42E, Table 3) suggests that maximum growth of branches had been obtained at all CO_2

TABLE 2.41. Mean total dry weight of soybean plants grown at all nitrogen levels at CO₂ concentrations of 400, 650, or 900 $\mu\text{L L}^{-1}$.

$\mu\text{L L}^{-1}$	Days of nitrogen treatment					
	0	4	7	11	14	18
	(g DW plant ¹)					
400	2.31a*	3.94a	5.76a	9.53a	15.23a	25.38a
650	2.32 (100%)**	5.15b (131%)	7.20b (125%)	11.33gb (119%)	15.01a (98%)	25.39a (100%)
900	3.21 (139%)	5.32b (135%)	8.18c (142%)	13.76c (145%)	18.05b (119%)	29.99b (118%)

* Means within a column followed by a different letter are significantly different at the 95% level of probability according to Scheffe's multiple comparison method.

** Percentage of dry wieght at 400 $\mu\text{L L}^{-1}$ CO₂.

TABLE 2.42. Mean total dry weight of soybean plants exposed to CO₂ concentrations of 400, 650, or 900 $\mu\text{L L}^{-1}$ during the pre-N-treatment period.

DmL L ⁻¹ CO ₂	Days after transplanting*					
	0	3	7	10	14	16
	(g DW plant ⁻¹)					
400	0.115a**	0.197a	0.315a	0.711a	1.579a	2.312a
650	0.115a (100%)*	0.191a (97%)	0.354a (112%)	0.771a (108%)	1.568a (99%)	2.320a (100%)
900	0.115a (100%)	0.540b (274%)	0.713b (226%)	0.770a (108%)	2.150b (136%)	3.214b (139%)

* At Day 0, 4-d old seedlings were transplanted into hydroponic culture system at respective CO₂ concentrations.

** Means within a column followed by a different letter are significantly different at the 95% level of probability according to Scheffe's multiple comparison method.

*** Percentage of dry weight at 400 $\mu\text{L L}^{-1}$.

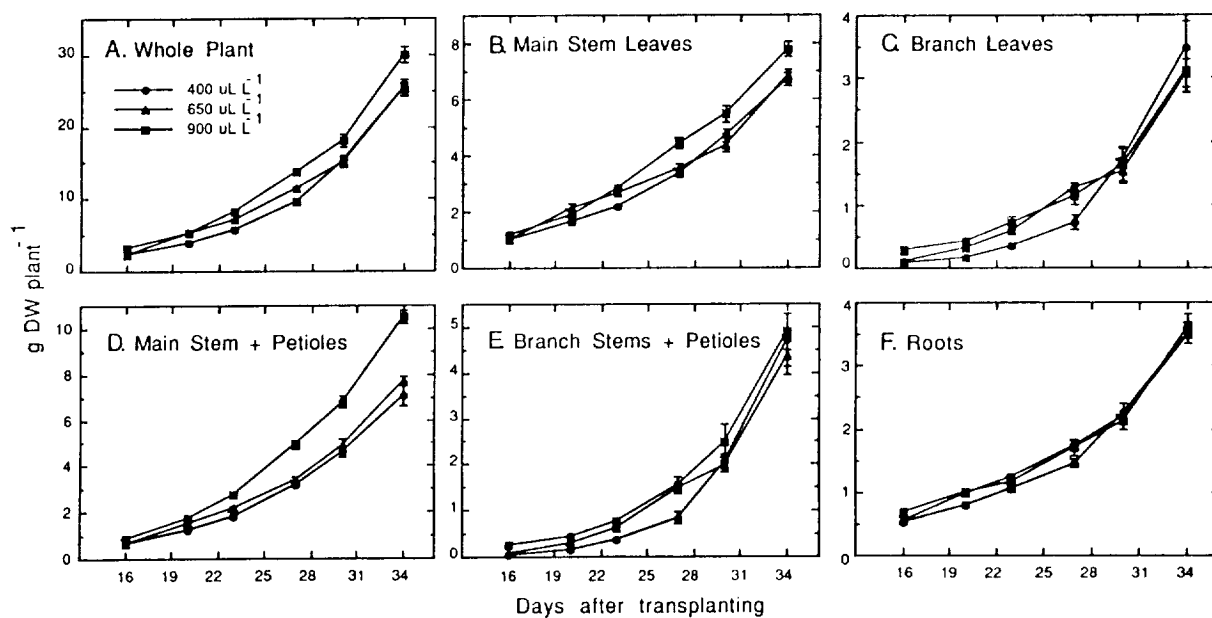


FIGURE 2.42 Dry weight of whole-plants or plant parts of soybean plants at all nitrate concentrations (i.e., 0.5, 1.0, 5.0, and 10.0 mM, combined) at CO₂ concentrations of 400, 650, or 900 μL L⁻¹ during an 18-d N-treatment period. Bars indicate ± 1 s.e.; n=3 for Day 0 data points and n=12 for all other data points.

levels. This pattern of an initial stimulation of growth, which diminishes with time, was also apparent in growth parameters of the whole plant (Fig. 2.42A, Tables 2.41, 2.43). These results indicate that elevated CO_2 levels may result in an initial growth enhancement by promotion of meristematic activity which increases sink capacity. The rate of meristematic activity, however, is finite. Once the limits are reached, and when photosynthetic capacity is not limited by other external factors, the potential for plant response to CO_2 enrichment becomes sink limited and growth at high CO_2 levels declines to rates similar to, or apparently slightly less than, that at a lower CO_2 level. The time-limited response of growth to CO_2 enrichment does not appear to be feed-back inhibition of photosynthesis, since at the higher CO_2 levels there was no evidence of an increase in C:N ratio of leaves (data not shown) as would be expected with a build-up of starch or soluble carbohydrates. The temporal effect of CO_2 levels on enhancement of growth requires that increases in dry matter accumulation of plants grown at elevated CO_2 should be considered in reference to the growth phase of the plant and the length of time of exposure to the elevated CO_2 conditions.

Regardless of level of atmospheric CO_2 concentration, dry matter accumulation, total N content (Table 2.44), and leaf area (Fig. 2.41) did not increase with increases in NO_3^- concentration in the flowing-solution culture. This indicates that the response of the plants to CO_2 enhancement was not limited by external concentration or availability of nitrogen. Such a limitation has been suggested in other stu-

TABLE 2.43 Mean leaf area of main stem, branch stems, and whole plant of soybean plants grown at all nitrogen levels and at CO₂ concentrations of 400, 650, or 900 $\mu\text{L L}^{-1}$.

$\mu\text{L L}^{-1}$	Days of nitrogen treatment					
	0	4	7	11	14	18
Main stem leaf area ($\text{m}^2 \text{ plant}^{-1}$)						
400	0.035a*	0.060a	0.087a	0.138a	0.186a	0.258a
650	0.035a (108%)	0.079b (130%)	0.111b (128%)	0.159b (115%)	0.203a (109%)	0.291b (113%)
900	0.044a (127%)	0.079b (130%)	0.130c (150%)	0.196c (142%)	0.243b (131%)	0.345c (134%)
Branch stems leaf area ($\text{m}^2 \text{ plant}^{-1}$)						
400	0.003a	0.008a	0.019a	0.045a	0.099a	0.195a
650	0.006a (199%)	0.017b (222%)	0.036b (181%)	0.085b (190%)	0.102a (102%)	0.209a (107%)
900	0.010a (292%)	0.019b (250%)	0.041b (212%)	0.074b (165%)	0.109a (110%)	0.196a (100%)
Whole plant leaf area ($\text{m}^2 \text{ plant}^{-1}$)						
400	0.038a	0.068a	0.106a	0.183a	0.285a	0.453a
650	0.043a (112%)	0.097b (142%)	0.146b (138%)	0.243b (133%)	0.304ab (107%)	0.500ab (110%)
900	0.054b (141%)	0.099b (145%)	0.172c (162%)	0.270b (148%)	0.353b (124%)	0.542b (120%)

*Means within a column of the same group followed by a different letter are significantly different at the 95% level of probability according to Scheffe's multiple comparison method.

**Percentage of leaf area at 400 $\mu\text{L L}^{-1}$ CO₂.

dies (5,10,52,84) which used solid substrates as culture media. We propose that the differences between our results and those of previous studies are due to the physical parameters which make NO_3^- available to the root surfaces in the two culture systems. In flowing-solution culture, NO_3^- is uniformly distributed in the culture solution and is constantly resupplied to the root surface for uptake independently of plant processes. For plants grown on solid media, however, NO_3^- is not uniformly distributed in the rooting medium, and its availability at the root surface is dependent primarily on transpiration-driven mass flow of the solution (6). In this study (Fig. 2.43), as in others (1,32,52), stomatal resistance increased with increasing concentration of CO_2 . Presumably, a decrease in the transpiration rate was associated with the increase in stomatal resistance. Thus, previously reported requirements for increased levels of NO_3^- to attain maximal response of plants grown in solid media at elevated concentrations of CO_2 may be the result of decreased transpiration and not due to direct effects of CO_2 on nitrogen metabolism.

In further support of our supposition, that plants grown in solid media at elevated CO_2 are N-limited due to nitrogen availability to the root, is the observation that most studies which report a response to nitrogen at elevated CO_2 levels also show increased root:shoot ratio (10,63,67) and increased C:N ratios in leaves as indicated by increases in levels of nonstructural carbohydrates and decreases in total nitrogen concentration of tissues (2,84). Both of these responses are symptoms of nitrogen deficiency (79). Whereas in the

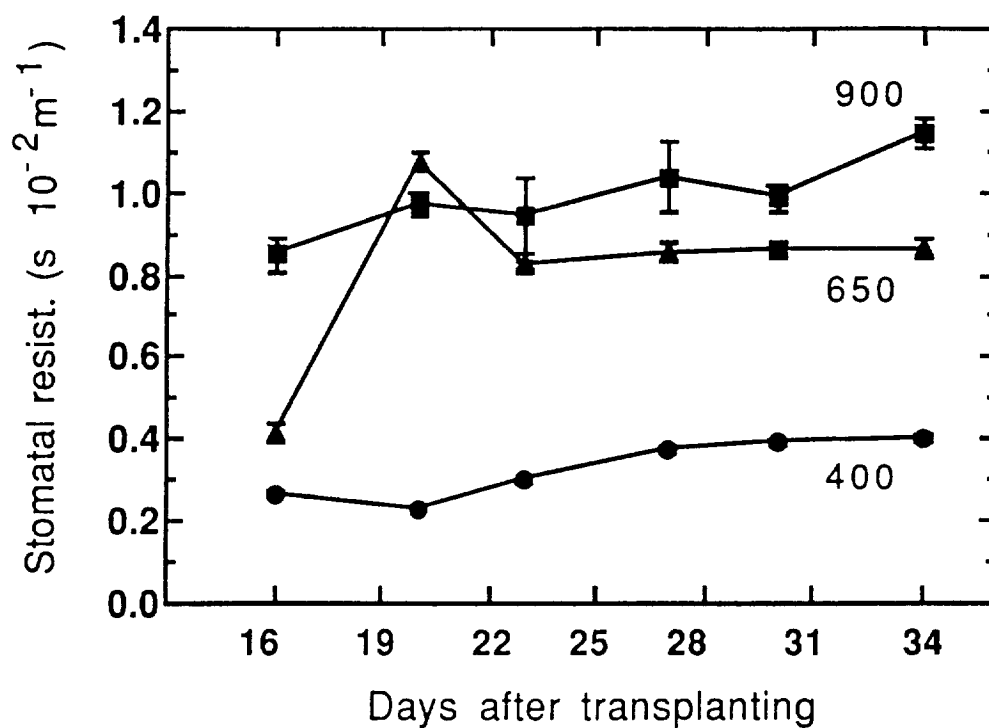


FIGURE 2.43 Stomatal resistance of the second-youngest, fully-expanded leaf of soybean plants grown at all nitrate concentrations (i.e., 0.5, 1.0, 5.0, and 10.0 mM, combined) at CO_2 concentrations of 400, 650, or 900 $\mu\text{L L}^{-1}$ during an 18-d N-treatment period. Bars indicate ± 1 s.e.; $n=3$ for Day 0 data points and $n=12$ for all other data points.

present study, plants grown at all NO_3^- levels at elevated CO_2 actually had lower root:shoot ratios (Table 2.45) with no increase in root dry weight (Fig. 2.42E) and no increase in C:N ratios of shoot tissues (data not shown). All these characteristics indicate that plants grown in hydroponic culture were not nitrogen limited at any of the levels of NO_3^- in solution.

It might be argued that the lack of an effect of NO_3^- concentration on plant growth at high CO_2 concentrations was the result of plant growth being limited by some other factor, such as PPFD, which was $700 \mu\text{mol m}^{-2}\text{s}^{-1}$ in this study. However, this does not appear to be the case in this study as the relative growth rate (RGR) of plants over the N-treatment period were 0.124 to $0.133 \text{ g g}^{-1}\text{d}^{-1}$. This is the same range of RGR calculated from data of Cure et al. (10) for soybeans of the same growth phase and grown under elevated CO_2 at PPFD of $1000 \mu\text{mol m}^{-2}\text{s}^{-1}$. Also, the C:N ratios of tissues of plants grown at all CO_2 concentrations did not show any decrease with increases in NO_3^- concentrations which might be expected if light, and hence CO_2 fixation, were limiting.

2.5 Enhancement of root respiration during nitrate uptake.

The treatment combinations within a split-root hydroponic system (Table 2.51) provided three conditions for supply of reduced nitrogen to maize roots. The -N axes of treatments nnL+ and nnL- were dependent entirely upon recycling from a nonreplenished pool in the shoot with the size of the pool more restricted for nnL- than for nnL+.

TABLE 2.44 Mean root:shoot ratio of soybean plants grown at all nitrogen levels and at CO₂ concentrations of 400, 650, or 900 $\mu\text{L L}^{-1}$.

$\mu\text{L L}^{-1} \text{CO}_2$	Days of nitrogen treatment					
	0	4	7	11	14	18
	(g DW g ⁻¹ DW)					
400	0.317a*	0.252a	0.219a	0.179a	0.172a	0.170a
650	0.283a (89%)**	0.230ab (91%)	0.203a (93%)	0.181a (101%)	0.171a (99%)	0.169a (99%)
900	0.264a (83%)	0.223b (89%)	0.164b (75%)	0.141b (79%)	0.130b (76%)	0.147b (87%)

* Means within a column followed by a different letter are significantly different at the 95% level of probability according to Scheffe's multiple comparison method.

** Percentage of root:shoot ratio at 400 $\mu\text{L L}^{-1} \text{CO}_2$.

TABLE 2.45. Mean total nitrogen content of soybean plants grown at all nitrogen levels at CO₂ concentrations of 400, 650, or 900 $\mu\text{L L}^{-1}$.

$\mu\text{L L}^{-1}$ CO ₂	Days of nitrogen treatment					
	0	4	7	11	14	18
	(mg N plant ⁻¹)					
400	112.2b*	148.8a	220.9a	344.3a	536.4a	816.3a
650	101.9a (91%)**	200.7b (135%)	268.9b (122%)	406.3a (118%)	532.5a (99%)	834.2a (102%)
900	136.1b (121%)	211.6b (142%)	325.5c (147%)	517.9b (150%)	671.7b (125%)	1034.5b (127%)

*Means within a column followed by a different letter are significantly different at the 95% level of probability according to Scheffe's multiple comparison method.

**Percentage of total nitrogen content of 400 $\mu\text{L L}^{-1}$ CO₂.

TABLE 2.51. Summary of nutritional and irradiance variables for treatments.

Treatment	Treatment NO_3^- concentration		PPFD	Pretreatment NO_3^-	H_2PO_4^-
	Axis 1	Axis 2			
	(mol m ⁻³)		($\mu\text{mol m}^{-2}\text{s}^{-1}$)	(mol m ⁻³)	(mol m ⁻³)
NNL+	1.0	1.0	595	0.1	0.15
NnL+	1.0	0.0	595	0.1	0.15
NNl+	1.0	1.0	210	0.2	1.50
Nnl+	1.0	0.0	210	0.2	1.50
nnL+	0.0	0.0	595	0.1	0.15
nnL-	0.0	0.0	595	0.0	0.15

The -N axes of NnL+ and Nnl+ treatments also were dependent upon recycling from the shoot, but the shoot pool was continually replenished by translocation of nitrogen from the +N axes. The source of reduced nitrogen for +N axes directly exposed to exogenous NO_3^- (both axes of NNL+ and Nnl+ as well as the +N axis of NnL+ and Nnl+) included in situ reduction of absorbed NO_3^- (23) in addition to recycling from a continually replenished shoot pool. Availability of photosynthates for translocation to roots was restricted by decreased irradiance from $595 \mu\text{mol m}^{-2}\text{s}^{-1}$ (NNL+ and NnL+) to $210 \mu\text{mol m}^{-2}\text{s}^{-1}$ (Nnl+ and Nnl+).

The treatments resulted in roots with a wide range in mass, growth rate, proliferation of lateral branches and concentration of reduced nitrogen. Dry weights and contents of reduced nitrogen in roots increased during the treatment period for all treatments (data not shown). Dry weight at the end of the 8-d treatment period ranged from $15.2 \text{ mg axis}^{-1}$ (with a mean relative growth rate of roots (R_r) of $0.07 \text{ mg mg}^{-1}\text{d}^{-1}$ during the treatment period) for the -N axis of treatment Nnl+ to $82.4 \text{ mg axis}^{-1}$ (with R_r of $0.23 \text{ mg mg}^{-1}\text{d}^{-1}$) for the +N axis of treatment NnL+ (Table 2.52). In general, greater dry weights of roots and equal or greater R_r 's were produced for plants grown under high than low irradiance (cf. axes for treatment NNL+ and NnL+ with Nnl+ and Nnl+), for the +N axis of treatments in which only one of the axes received exogenous NO_3^- than for the +N axes of treatments in which both axes received exogenous NO_3^- (cf. +N axis of NnL+ with +N axes of NNL+ and Nnl+), and for the +N axis than for the -N axis of treatments

TABLE 2.52. Characteristics of development of root axes. Total dry weight of roots and density of branch roots on the portion of primary axis produced during first 4 d of treatment were determined on final day of the 8-d treatment period when plants were 19 to 24 d old. Concentration of reduced nitrogen was averaged over all sampling dates and \pm s.d. among dates is reported. Mean relative growth rates of roots (R_r) and extension rate of primary axis (ER) were calculated as regression coefficients over the treatment period. ns, *, and ** indicate differences between axes not significant and significant at 0.05 and 0.01 levels of probability, respectively, according to a 2-tailed standard Student's t test.

Treatment	Axis	Total dry weight (mg axis ⁻¹)	Reduced nitrogen (mg g ⁻¹ d.w.)	R_r (mg m ⁻¹ g ⁻¹ d ⁻¹)	ER (cm d ⁻¹)	Density of branches (no. cm ⁻¹ axis ⁻¹)
NNL+	+N	41.4	32.9 \pm 4.8	0.24	4.4	11.8
	+N	38.9 (ns)	33.0 \pm 4.7 (ns)	0.23 (ns)	4.5 (ns)	12.3 (ns)
NnL+	+N	82.4	33.8 \pm 3.9	0.23	4.8	11.4
	-N	35.3 (**)	22.9 \pm 1.3 (**)	0.12 (**)	3.9 (ns)	8.5 (**)
NNl+	+N	39.3	25.2 \pm 6.3	0.18		
	+N	31.3 (*)	22.1 \pm 4.0 (ns)	0.14 (ns)		
Nnl+	+N	55.1	29.0 \pm 10.0	0.23		
	-N	15.2 (**)	25.1 \pm 15.4 (**)	0.07 (**)		
nnL+	-N	59.2	17.5 \pm 8.5	0.21	4.7	8.4
	-N	63.3 (ns)	16.9 \pm 7.4 (ns)	0.21 (ns)	4.8 (ns)	8.0 (ns)
nnL-	-N	25.7	9.9 \pm 1.4	0.10	2.9	5.3
	-N	20.1 (ns)	9.0 \pm 1.7 (ns)	0.08 (ns)	2.3 (ns)	5.0 (ns)

NnL+ and Nnl+. For treatments in which exogenous NO_3^- was supplied to neither axis during the treatment period (nnL+ and nnL-), final dry weights and R_r 's were greater when NO_3^- had been supplied to the axes during the 4-d pretreatment (nnL+) than when no NO_3^- was supplied during pretreatment (nnL-). The final dry weights and R_r 's of roots when neither axis was supplied with NO_3^- during the treatment period, but had received NO_3^- during pretreatment (nnL+), were greater than those of the -N axis of than, and their R_r 's were similar to, those of the +N axes of treatment NNL+. The increase in dry weights for these low N roots was attributable principally to extension of primary axes since proliferation of lateral branches was inhibited (Table 2.52). Density of first order laterals was greater than 11 cm^{-1} of primary axis for the +N axes of NNL+ and NnL+, but was 8.4 cm^{-1} or less for the primary axes of nnL+ and nnL-. Other indications of diminished proliferation for -N axes were decreased length, thickness and absence of second order branches relative to the +N axes (data not shown).

Respiration rate of roots is enhanced by an increase in the supply of nitrogen (7,12,23,61,62). Over a limited range in concentration of reduced nitrogen in tissue, the response in respiration can be described adequately as linear (27), but over a more extended range, including concentrations approaching the minimum for support of respiratory activity, the rapid decreases in respiration rate are not approximated well by a straight line. The description of specific rate of respiration as a rectangular hyperbolic function of con-

centration of reduced nitrogen (solid line in Fig. 2.51) accounts for 82% of the variation in specific rates of respiration and approximates both the rapid changes in respiratory response at low concentrations and the approach to a maximum rate at high concentrations. The lowest specific rates of respiration occurred in roots entirely dependent on nitrogen recycled from the shoots and initiating the fewest lateral branches (open symbols in Fig. 2.51, Table 2.52). The highest respiration rates were associated with +N roots of plants grown at the higher irradiance level (NNL+ and NnL+). These latter axes, which were actively involved in uptake and assimilation of exogenously supplied NO_3^- , had the greatest proliferation of lateral branches (Table 2.52). Intermediate specific rates of respiration occurred for +N roots of plants grown at the lower irradiance level (NNl+ and Nnl+), but carbohydrate availability in these roots presumably was limited relative to that in roots of plants grown at the higher irradiance.

Since general positive correlations exist between both respiration rate and nitrogen concentration (35,36) and between nitrogen concentration and relative growth rate (27), part of the enhanced specific rate of respiration associated with concentration of reduced nitrogen may be attributed to the acceleration of dry matter accumulation, or relative growth rate (RGR_r), in roots that occurred as concentrations of reduced nitrogen in the roots increased. However, since dry matter accumulation was not uniformly proportional to accumulation of reduced nitrogen among all roots (Table 2.52), only 25%

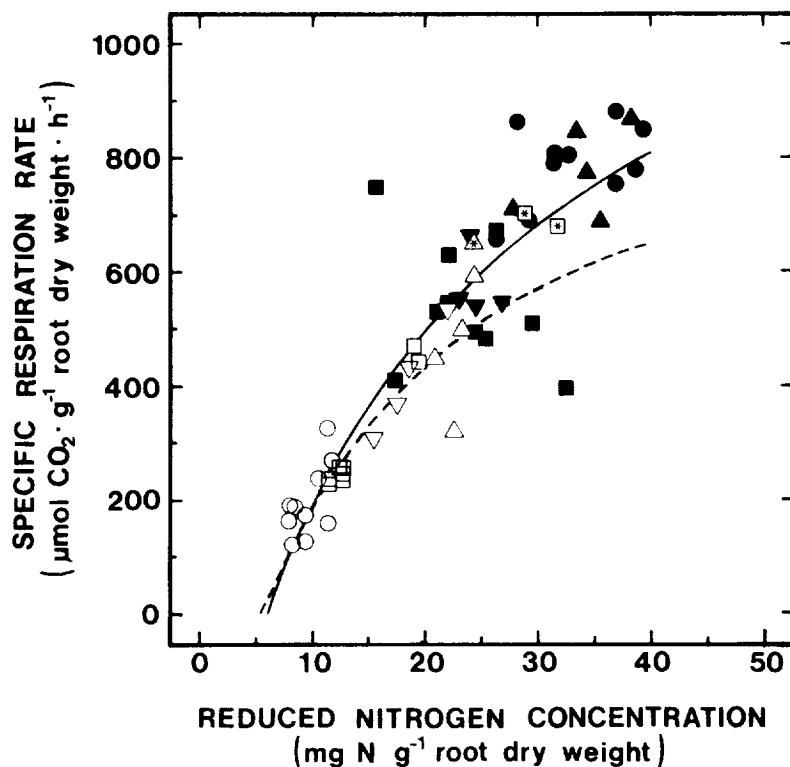


FIGURE 2.51 Dependence of specific rate of respiration for roots on concentration of reduced nitrogen in roots. Closed symbols represent measurements of +N axex in treatments NNL+ (●), NN1+ (■), NnL+ (▲), and Nn1+ (▼). Open symbols represent measurements of -N axes in treatments NnL+ (△), Nn1+ (▽), nnL+ (□), and nnL- (○). See Table 2.51 for a description of treatments. The solid line corresponds to the rectangular hyperbolic function $R_s = [1402 (RN - 6.0)] / [25.2 + (RN - 6.0)]$, where R_s is specific respiration rate and RN is concentration of reduced nitrogen, fitted by a least squares technique to all observations with $R^2 = 0.82$. The dashed line corresponds to the rectangular hyperbolic function $R_s = [1024 (RN - 5.2)] / [20.0 + (RN - 5.2)]$ fitted only to observations of axes receiving $0.0 \text{ mol m}^{-3} \text{ NO}_3^-$ (open symbols), but excluding the observations designated by * for which NO_3^- from pretreatment solutions had not been depleted, with $R^2 = 0.83$.

of the variation in RGR_r could be accounted for by differences in concentration of reduced nitrogen (Fig. 2.52B). Furthermore, description of specific rate of respiration as a function of RGR_r (Fig. 2.52A) does not account for respiratory activity related to either nitrogen assimilation or the enhanced metabolic activity of root apices associated with proliferation of lateral branches for axes assimilating exogenous NO_3^- ; thus, only 30% of the variation in specific rate of respiration was explained by differences in RGR_r .

An estimate of the respiration attributable directly to active uptake and assimilation of NO_3^- and indirectly to the stimulation of lateral proliferation can be derived by a comparison of relationships between specific rate of respiration and concentration of reduced nitrogen for all axes (solid line in Fig. 2.51) and for only those axes not receiving exogenous NO_3^- (open symbols and dashed line in Fig. 2.51). A comparison between these relationships at a concentration of reduced nitrogen of 23 mg g^{-1} dry weight, below which initiation of lateral branches was restricted (Table 2.52), indicates that specific rate of respiration was enhanced by about 17% for roots absorbing exogenous NO_3^- . Although this value falls within the lower range of values reported (75,76,78) for the proportion of root respiration expended for ion uptake, only a small fraction of the increase in specific respiration presumably can be attributed directly to uptake of NO_3^- . The uptake rates of NO_3^- were not directly measured in this study. In a previous investigation (23), however, the average

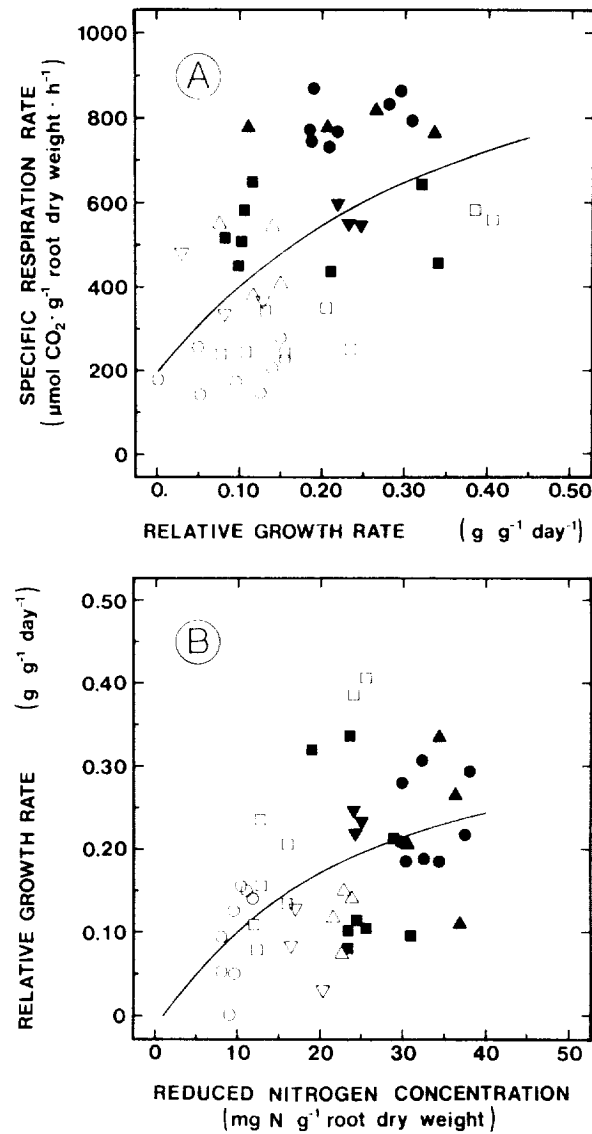


FIGURE 2.52 Dependence of (A) specific rate of respiration on relative growth rate of roots and (B) relative growth rate of roots on concentration of reduced nitrogen in roots. Symbols are as described for Fig. 2.51. The solid lines correspond to the rectangular hyperbolic functions $R_s = [1093 \text{ RGR}_r / (0.42 + \text{RGR}_r)] + 198$, with $R^2 = 0.30$, and $\text{RGR}_r = [0.43 (\text{RN} - 0.9)] / [29.0 + (\text{RN} - 0.9)]$, with $R^2 = 0.25$, where R_s is specific rate of respiration, RGR_r is relative growth rate of roots, and RN is concentration of reduced nitrogen, fitted by a least squares technique.

NO_3^- -uptake rate when only one axis in a split-root system received exogenous NO_3^- was 1.6 times the rate for an axis when both axes were supplied with exogenous NO_3^- . The average specific rates of respiration for these axes were similar and did not reflect the difference in uptake rates. Thus, the enhanced specific rate of respiration for roots supplied with exogenous NO_3^- apparently can be attributed primarily to the energy utilized to support processes such as in situ assimilation of absorbed NO_3^- and initiation of lateral branches rather than for direct support of ion uptake.

While its quantitative contribution cannot be determined from results of the study, NO_3^- assimilation apparently contributed to the enhanced specific rate of respiration for the NO_3^- -fed roots and, based on previous findings (23), provided the initial stimulation in sink activity for enhanced partitioning of photoassimilates to axes growing in the presence of exogenous NO_3^- . Although reduced nitrogen readily cycles between shoot and roots (8,23,79,86) and can account for the increase during treatment in the content of reduced nitrogen of axes not absorbing NO_3^- (Table 2.52), part of the increased concentration of reduced nitrogen in roots supplied with exogenous NO_3^- (Fig. 2.51) apparently is derived from in situ reduction (23). While the correlation between actual NO_3^- reduction and in vitro NO_3^- activity cannot be presumed to be quantitative, NO_3^- reductase activity increases from basipetal to apical regions of roots (23,50,54) where lateral primordia are initiated (15,23,25). Differences in concentration of reduced nitrogen along the length of +N axes (23) followed the same gradient as specific rate of respiration (60) and

NO_3^- reductase activity (23) with the greatest concentration in the apex. The resulting increase in availability of reduced nitrogen to the apex of the +N axes, along with the increased availability of photoassimilates, were proposed as processes involved in proliferation of lateral branches (23). We thus suggest that a major portion of the enhanced specific rate of respiration as a response to increased concentrations of reduced nitrogen in roots is associated with increased respiratory activity of apices involved in proliferation of lateral roots which is stimulated by enhanced availability of reduced nitrogen for roots actively absorbing NO_3^- . We also suggest that interpretation of respiratory activity of roots in response to nitrogen uptake and assimilation should take into consideration the morphological response of roots to nitrogen supply.

SUMMARY OF PROPOSED RESEARCH DURING THIRD YEAR OF FUNDING

3. Objectives

3.1 Effects of nitrate concentration in nutrient solution on nitrogen uptake rates and reproductive growth of soybean

In our previous studies on the effects of NO_3^- at concentrations between 0.5 and 10.0 mM in nutrient solution, we found that during the vegetative growth stage the total accumulation of nitrogen and dry matter in the plant was not altered over the 0.5 to 10.0 mM range. However, during reproductive growth, seed fill of soybean represents an intense sink for nitrogen as well as photosynthates. There are reports in the literature that under field conditions the demand by seed for nitrogen and photosynthates pre-empts the ability of the plant to maintain sufficient translocation of carbohydrates to the roots for support of continued uptake of NO_3^- and the ability of the roots to provide adequate nitrogen to leaves to maintain photosynthetic rates. This will be evaluated in hydroponic culture by following NO_3^- uptake by roots from nutrient solutions containing 1.0 and 10.0 mM NO_3^- , dry matter and nitrogen accumulation and partitioning within the plant, and seed growth and yield during reproductive stages.

3.2 Consequences of nitrogen availability from nitrate, ammonium, and mixed sources of nitrate and ammonium on reproductive growth of soybean.

In our previous studies of the effects of NO_3^- and NH_4^+ at varying levels of pH on soybean growth, we found that plants which were grown on combined sources of NO_3^- and NH_4^+ at pH 6.0 accumulated greater concentrations of total nitrogen in their tissues than did plants grown on NO_3^- or NH_4^+ alone. The data also suggested that the increased nitrogen accumulated by the plant tissues was as vacuolar NO_3^- . These findings raise the question whether this increased accumulation of plant nitrogen during vegetative growth can translate into increases in reproductive growth and yield. This question will be addressed by following nitrogen uptake and growth of soybean plants provided with nitrogen sources of NO_3^- , NH_4^+ , and NO_3^- plus NH_4^+ through vegetative and reproductive growth.

3.3 Whole-plant regulation of nitrate uptake rate.

Previous studies in this project have demonstrated that a cyclic variation occurs in the daily uptake of NO_3^- by soybean plants in hydroponic culture. The uptake rates oscillated between maxima and minima rates with a 3 to 5 d periodicity. This was believed to be the result of a variation in plant NO_3^- demand which is hypothesized to be related to the interval between emergence of successive leaves. To test this hypothesis we will grow plants from germination at lower aerial temperature (18/14°C) than in previous studies (26/22°), which will increase the interval of leaf emergence and, we propose, will similarly affect the period of cyclic uptake of NO_3^- from the nutrient solution.

3.4 Diurnal patterns of nitrogen uptake.

Purchases of equipment are underway to upgrade the hydroponic system to continuously monitor concentrations of NO_3^- , H_2PO_4^- , SO_4^{2-} , Cl^- , and NH_4^+ in nutrient solutions. After installation of the system to continuously monitor anion and cation concentrations in the nutrient solutions, a study will be undertaken to investigate the diurnal patterns in nitrogen uptake of soybean plants from sources of NO_3^- and NH_4^+ . A constant root-zone temperature of 24°C and diurnal changes in temperature of $29/21^\circ\text{C}$ in the root-zone also will be used to investigate the effect of temperature on the diurnal pattern of nitrogen uptake. Such studies of diurnal patterns of nitrogen uptake are rare, and a long-term study of these patterns as plants develop may be unique. Such information will provide some basic information on nitrogen uptake by plants which in the past has been generally ignored or which has been addressed by assumptions.

4. Procedures

4.1 Effects of nitrate concentration in nutrient solution on nitrogen uptake rates and reproductive growth of soybean.

Non-nodulated soybean seedlings initially will be grown in complete nutrient solutions containing 1.0 mM NO_3^- at pH 6.0. Aerial temperature will be $26/22^\circ\text{C}$, solution temperature will be 24°C , aerial CO_2 will be $400 \mu\text{L L}^{-1}$, and PPFD during a 9-h day will be $700 \mu\text{mol m}^{-2}\text{s}^{-1}$. Plants will be maintained in a vegetative state until expansion of the fourth trifoliolate leaf by a 3-h interruption in the middle of the 15-h dark period with incandescent lamps. Upon expan-

sion of the fourth trifoliate, the dark interruption will be discontinued to promote reproductive development with about 80 pods per plant. Other environmental conditions, except for NO_3^- concentration in nutrient solution, will be continued from vegetative development. The NO_3^- levels in solution will be changed to 1.0 and 10.0 mM for the remainder of reproductive growth. Daily uptake of NO_3^- will be monitored by ion chromatography and NO_3^- will be resupplied to solutions to maintain concentrations at 1.0 and 10.0 mM. Plants will be harvested at 4 to 7 d intervals until seed maturity for determination of dry matter and nitrogen accumulation and partitioning among vegetative and reproductive tissues.

4.2 Consequences of nitrogen availability from nitrate, ammonium, and mixed sources of nitrate and ammonium on reproductive growth of soybean.

Environmental conditions, except for nitrogen source and concentration in nutrient solutions, will be the same as in the preceding study. Four nitrogen treatments will be established: 1:0 mM NO_3^- throughout growth, 1.0 mM NH_4^+ throughout growth, 0.5 mM NO_3^- plus 0.5 mM NH_4^+ throughout growth, and 0.5 mM NO_3^- plus 0.5 mM NH_4^+ during vegetative growth which will be changed to 1.0 mM NH_4^+ during reproductive growth. Daily uptake rates of NO_3^- and NH_4^+ will be monitored by ion chromatography from replenished solutions. Plants harvested at 4 to 7 d intervals will be analyzed for NO_3^- concentrations as well as total nitrogen and dry matter accumulation and seed yields.

4.3 Whole-plant regulation of nitrate uptake rate.

Non-nodulated soybean seedlings initially will be grown in complete nutrient solutions containing 1.0 mM NO_3^- at pH 6.0 until emergence of the third trifoliolate leaf. Aerial temperature will be $18/14^\circ\text{C}$, solution temperature will be 22°C , aerial CO_2 will be $400 \mu\text{L L}^{-1}$, and PPFD during a 9-h day will be $700 \mu\text{mol m}^{-2}\text{s}^{-1}$. Plants will be maintained throughout the study in a vegetative state by a 3-h interruption in the middle of the 15-h dark period with incandescent lamps. When the third trifoliolate leaf emerges, plants will be divided between two nitrogen sources of 1.0 mM NO_3^- and 1.0 mM NH_4^+ . Other environmental conditions will be continued. Daily uptake rates of NO_3^- and NH_4^+ will be monitored by ion chromatography from replenished solutions. Plants will be harvested at 3 to 4 d intervals for determination of number of leaves, total nitrogen and dry matter accumulation, and NO_3^- concentrations. Additionally, one subset of plants for each treatment will be used for nondestructive determination of time and rate of emergence of individual leaves on the mainstem and on each branch stem.

4.4 Diurnal patterns of nitrogen uptake.

Non-nodulated soybean seedlings initially will be grown in complete nutrient solutions containing 1.0 mM NO_3^- at pH 6.0 until emergence of the third trifoliolate leaf. Aerial temperature will be $26/22^\circ\text{C}$, solution temperature will be 24°C , aerial CO_2 will be $400 \mu\text{L L}^{-1}$, and PPFD during a 9-h day will be $700 \mu\text{mol m}^{-2}\text{s}^{-1}$. Plants will be maintained throughout the study in a vegetative state as in the preceeding study. When the third trifoliolate leaf emerges, the

plants will be divided into two groups that receive either 1.0 mM NO_3^- or 1.0 mM NH_4^+ . Additionally, half of the plants receiving each nitrogen source will continue to be grown at a constant solution temperature of 24°C, and the other half will be changed to a solution temperature that has a diurnal variation of 29/21°C. The NO_3^- and NO_4^+ uptake rates will be monitored at 1-h intervals by ion chromatography from replenished solutions. Plants will be destructively sampled at 3 to 4 d intervals for determination of total nitrogen and dry matter accumulation.

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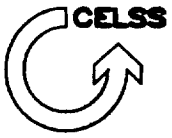
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16. Abstract <p>An important feature of controlled-environment crop production systems such as those to be used for life support of crews during space exploration is the efficient utilization of nitrogen supplies. Making decisions about the best sources of these supplies requires research into the relationship between nitrogen source and the physiological processes which regulate vegetative and reproductive plant growth. This paper reports on work done in four areas within this research objective: 1) experiments on the effects of root-zone pH on preferential utilization of NO₃⁻ versus NH₄⁺ nitrogen, 2) investigation of processes at the whole-plant level that regulate nitrogen uptake, 3) studies of the effects of atmospheric CO₂ and NO₃⁻ supply on the growth of soybeans, and 4) examination of the role of NO₃⁻ uptake in enhancement of root respiration.</p>			
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